

SHORT COMMUNICATION

Analysis of Respiratory Viral Infections Detected Using Multiplex Real-Time PCR in Hwaseong, Korea from 2013 to 2015

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SUMMARY

Background: We investigated the incidences and age-related/seasonal variations of respiratory virus infections in a regional area in Korea.

Methods: A total of 3,467 respiratory specimens from patients with acute respiratory infection symptoms in a teaching hospital were tested for respiratory viruses during 2013 - 2015.

Results: At least one virus was detected in 2,561 of the 3,467 specimens (73.9%), and 706 patients (20.4%) were positive for two or more viruses. The most frequently detected viruses were rhinovirus (23.9%), respiratory syncytial virus B (15.5%), and adenovirus (12.5%). Most of the patients (with and without a detected virus) were children. Young children (< 5 years old) were significantly more likely to have two or more viruses compared to older individuals ($p < 0.0001$). Most viruses exhibited seasonal variations.

Conclusions: This study revealed the incidence of respiratory virus infections. These findings can enhance our understanding of the distribution of respiratory viruses according to patient age and season.

(Clin. Lab. 2017;63:xx-xx. DOI: 10.7754/Clin.Lab.2016.161118)

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KEY WORDS

respiratory viruses, multiplex PCR

INTRODUCTION

Respiratory virus (RV) infections are the most common infectious diseases worldwide [1-3], and the causative viruses are numerous [4-6]. As multiplex PCR-based methods have become widely available in clinical laboratories, the distributions of RV infections and seasonal variation have been revealed in many reports [4-6]. This unveiling of RV infection epidemiology is crucial for disease monitoring, infection control, and establishing laboratory plans for diagnosing RV infections.

Although the frequencies and seasonal variations of RV infections in Korea have been described in several reports [4-6], there is no report on the frequencies of RV infections determined using multiplex real-time PCR technique in Korea or on the RV frequencies in Hwaseong, which is a newly developed and growing city located south of Seoul and may have a different pattern of RV infection.

Therefore, this retrospective observational study reports the age-related and seasonal frequencies of RVs over a recent 3-year period at a teaching hospital located in Hwaseong, a newly developed city in Korea.

MATERIALS AND METHODS

Specimens

The specimens were collected at Dongtan Sacred Heart Hospital, Korea, between January 2013 and December 2015. During the study period, nasopharyngeal swabs were obtained from patients who were suspected of having acute respiratory infections with fever of $\geq 38^\circ\text{C}$ and at least one of following symptoms: cough, sore throat, rhinorrhea and/or nasal congestion. Multiplex real-time PCR for detecting RVs were tested on these samples. This study was conducted retrospectively under the approval of the institutional review board of Dongtan Sacred Heart Hospital (2015-069).

Multiplex real-time PCR

RNA was extracted from the specimens using the QIA-amp Viral RNA mini kit and the QIAcube platform (Qiagen, Hilden, Germany). The Anyplex II RV16 Kit (RV16; Seegene, Korea) was used to detect the RVs according to manufacturer's instructions. This kit is a multiplex real-time PCR platform that is designed to simultaneously identify 16 common RVs: human adenovirus (AdenoV); human bocavirus (BocaV); human coronaviruses 229E (CoV 229E), NL63 (CoV NL63), and OC43 (CoV OC43); human enterovirus (EnterovV); influenza viruses A and B (Inf A and Inf B); human metapneumovirus (MPV); human parainfluenza viruses 1 - 4 (PIV 1, PIV 2, PIV 3, and PIV 4); human rhinovirus (RhinoV); and human respiratory syncytial viruses A and B (RSV A and RSV B).

Statistics

All statistical analyses were performed using SPSS software (version 23; SPSS Inc., Chicago, IL, USA). P-values of < 0.05 were considered statistically significant.

RESULTS

During the study period, 3,467 specimens from 3,181 patients were sent to the laboratory for testing. Viruses were detected in 2,561 of the 3,467 specimens (73.9%), and 706 cases (20.4%) were positive for two or more viruses. Two viruses were co-detected in 604 patients (17.4%), 96 patients (2.8%) had three viruses, and 6 patients (0.2%) had four viruses.

The age ranges were 0 - 91 years (median: 1.0 year) for all patients and 0 - 82 years (median: 1.0 year) for the virus-positive patients. The most frequently detected viruses were RhinoV (23.9%), RSV B (15.5%), and AdenoV (12.5%) (Table 2). Most of the patients (with

and without detected viruses) were children, and younger children (< 5 years old) were significantly more likely to have multiple infections (two or more viruses), compared to older individuals ($p < 0.0001$). The positive rates also tended to be higher among young patients (Table 1).

Most viruses exhibited seasonal variations (Figure 1). BocaV and MPV exhibited peak incidences during spring season, while EnterovV and PIV were prevalent in summer season. During autumn AdenoV showed high positive rates, while CoV and Inf A/B exhibited peak incidences during winter season. However, RhinoV did not exhibit any obvious seasonal variations.

DISCUSSION

The present study revealed the incidence, age-specific variations, and seasonal variations of RV infections in a Korean city. Approximately 70% of the specimens tested positive for at least one RV, and 20.4% of the specimens tested positive for ≥ 2 RVs. The positive rate and co-infection rate of RVs in this study were higher compared to other previous reports [4]. This is probably related to the use of a more sensitive real-time multiplex PCR, rather than conventional multiplex PCR assay, in this study or to the characteristics of a newly developed city, which includes a large proportion of children, of which most are educated in community-based institutions.

Most RVs exhibited seasonal variations, although rhinovirus was detected throughout the year. Noh et al. reported an RV-positive rate of 52.1% (1,033/1,983), found that Inf A was the dominant agent among all age groups, and found that coinfection was identified in 5.6% of the cases (58/1,033) [4]. Another report regarding RV infections in Korea revealed higher incidences of RhinoV, AdenoV, RSV A, RSV B, and MPV among 2,800 pediatric patients, compared to among 763 adult patients, although Inf A was frequently detected among adults [7]. Our results also revealed that most of the target viruses were more frequently detected among children compared to among adults. This is probably related to the higher viral load and prolonged shedding of viruses among children [8]. Furthermore, Kim et al. performed a 5-year study in a Korean hospital and found that RSV was prevalent among ≤ 5 -year-old children, while Inf A was prevalent among > 5 -year-old patients [6]. However, they used an older version of the Anyplex assay which could not detect BocaV, MPV, and PIV 4. Thus, unlike the previous reports, the present study has several specific advantages: data from many specimens ($> 3,000$) were collected at one hospital during a relatively long period (3 years), and testing was performed using fresh specimens immediately after their collection. In this context, multiple freeze-thaw cycles can reduce the quality of the specimens [8], and some of the previous studies used stored specimens [4]. Similar results were also reported in Japan showing that in Japa-

Table 1. Positive rates (%) of respiratory viruses according to patients' age groups.

Age (N)	< 1 (1045)	1 (889)	2 (526)	3 (291)	4 (174)	5 - 10 (344)	11 - 20 (48)	21 - 40 (30)	41 - 60 (54)	> 60 (66)	Total (3467)
AdenoV	7.5	15.2	16.0	18.9	17.8	12.2	10.4	0.0	9.3	0.0	12.5
BocaV	4.9	11.5	5.3	2.7	2.3	1.5	0.0	0.0	1.9	0.0	5.7
CoV 229E/NL63	1.3	1.8	1.7	1.0	2.9	1.5	0.0	3.3	1.9	0.0	1.7
CoV OC43/HKU1	6.0	4.4	3.2	1.7	2.9	2.0	0.0	0.0	0.0	1.5	4.1
Enterov	2.8	4.2	3.0	6.5	4.0	4.1	4.2	0.0	1.9	0.0	3.6
Inf A	1.6	1.8	3.4	4.1	6.3	7.0	6.3	3.3	9.3	3.0	3.1
Inf B	0.9	2.4	2.1	4.1	4.6	6.4	8.3	3.3	3.7	1.5	2.6
MPV	4.5	8.4	10.1	12.0	6.9	1.5	2.1	3.3	5.6	0.0	6.7
PIV 1	2.6	5.8	6.3	4.1	2.9	1.5	2.1	0.0	0.0	0.0	3.9
PIV 2	0.4	1.1	1.0	2.7	1.1	0.6	2.1	0.0	0.0	1.5	1.0
PIV 3	3.2	4.5	2.7	1.0	1.7	0.3	2.1	0.0	0.0	0.0	2.7
PIV 4	2.1	3.3	3.8	1.7	2.9	2.3	0.0	0.0	1.9	0.0	2.6
RhinoV	24.8	27.1	26.4	24.4	19.0	19.8	18.8	3.3	11.1	4.5	23.9
RSV A	17.4	4.6	6.8	3.8	4.6	0.6	0.0	0.0	0.0	1.5	8.1
RSV B	23.3	18.2	16.5	6.5	5.2	3.8	2.1	0.0	1.9	0.0	15.5

Abbreviations: AdenoV - adenovirus, BocaV - bocavirus, CoV - coronavirus, Enterov - enterovirus, Inf - influenza, MPV - metapneumovirus, PIV - parainfluenza, RhinoV - rhinovirus, RSV - respiratory syncytial virus.

nese children with acute respiratory illnesses, RSV, RhioV, and MPV were prevalent and their co-infection rate was 9% [9]. Another study using viral culture and real-time PCR showed that RSV was the most frequently isolated virus in children, with the co-infection rate of 19.7% [10]. Since Korea and Japan are geographically close and have similar ethnic and social environments, this Korean study would be helpful to find RV infection and predict the disease prevalent in Japan.

Our results revealed clear seasonal variations in the incidences of RV infections, which support the current understanding that many RV infections have seasonality. However, there is no definite explanation for this seasonality. Some studies have suggested several hypotheses, such as changes in social contacts, pathogen stability in the environment, reduced host susceptibility during winter because of the reduced sunlight exposure, and periodic changes in viral pathogenicity that result from viral mutation [11]. For example, influenza seasonality in the winter might be attributed to the diminished daylight and resulting vitamin D deficiency [12, 13], temperature, and humidity that favor pathogen spread [14, 15], and/or air travel during the holidays [16, 17]. Therefore, additional research is needed to establish the cause(s) of RV infection seasonality.

Similar to previous studies, the present study used multiplex real-time PCR for RV detection, although this method has several inherent limitations. First, it cannot

differentiate between live pathogens and dead inactive viruses. Thus, as some viruses can be detected in asymptomatic individuals, it is difficult to determine whether an individual has an infectious disease or simple colonization. Second, the quality of the specimens can influence the assay's results. Nevertheless, this technique has many advantages over the traditional culture-based and/or direct fluorescent assays, as it requires less hands-on time and provides better detection capabilities. Therefore, multiplex real-time PCR has become a standard assay in many clinical laboratories [18, 19] and is suitable for our estimation of the incidences of RV infections.

CONCLUSION

We assessed the incidences of RV infections in Korea using a multiplex real-time PCR assay. More than 70% of the tested specimens had detectable viruses, and approximately 20% of the specimens had two or more viruses. Furthermore, the positive rate was significantly higher among children compared to among adults, and many pathogens exhibited seasonal variations. Therefore, our findings provide valuable information regarding the incidences of RV infections, which may be helpful for managing and controlling acute respiratory infections.

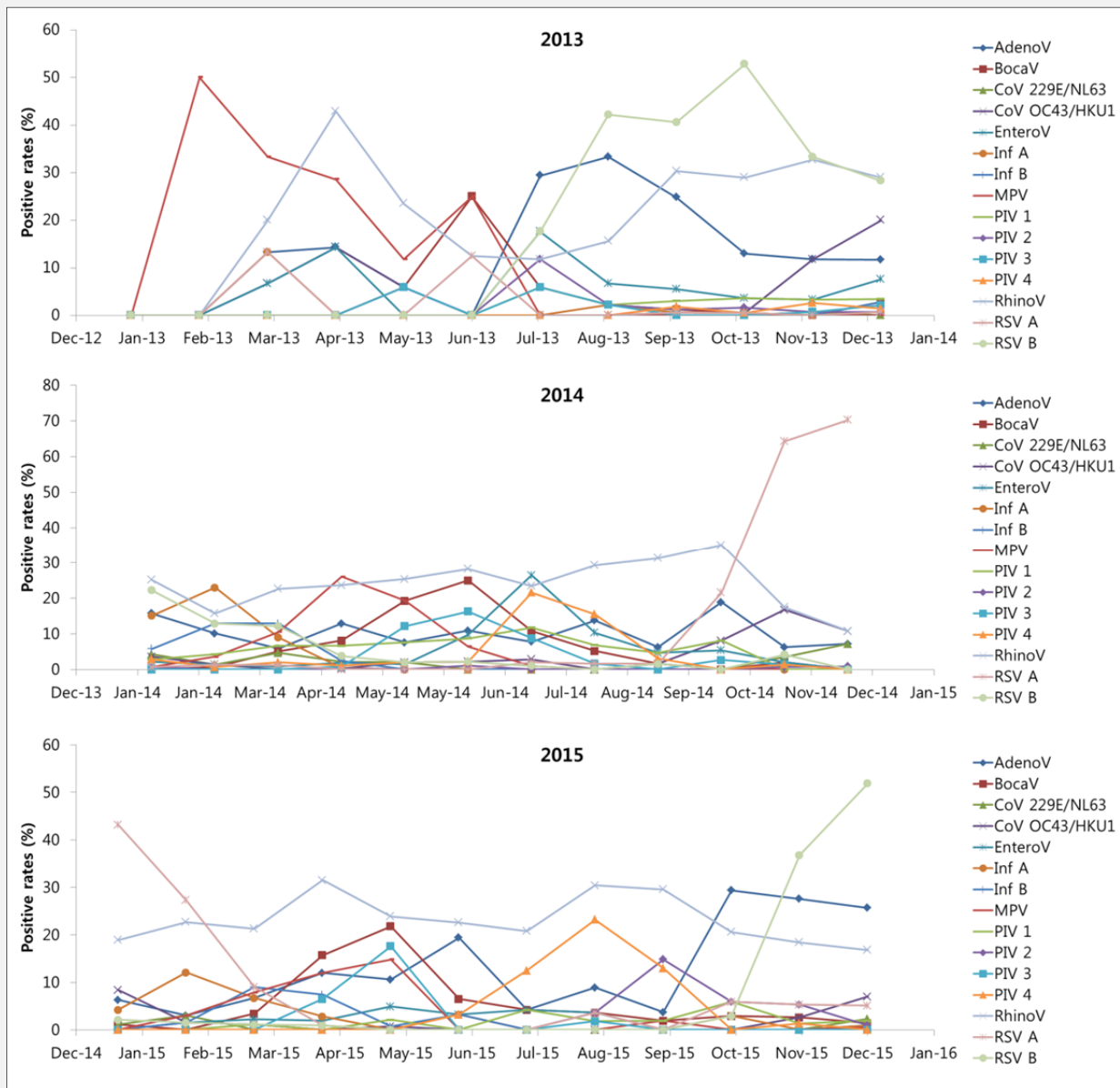


Figure 1. Positive rates (%) of respiratory viruses according to months in 2013 - 2015.

Abbreviations: AdenoV - adenovirus, BocaV - bocavirus, CoV - coronavirus, EnteroV - enterovirus, Inf - influenza, MPV - metapneumovirus, PIV - parainfluenza, RhinoV - rhinovirus, RSV - respiratory syncytial virus.

Acknowledgement:

The authors appreciate Ji Sun Noh for the excellent technical assistance.

Declaration of Interest:

No potential conflicts of interest relevant to this study were reported.

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